Chapter 6

Synthesis of 3-Mercaptobenzoic acid assisted ZnO nanostructures and its characterization along with antibacterial activity study

1.1 INTRODUCTION 125-127
1.2 EXPERIMENTAL PROCEDURE 128
6.3 RESULTS AND DISCUSSION 129-145
6.4 CONCLUSION 146
6.5 REFERENCES 147-148
6.1 Introduction

Nanoparticle metal oxides represent a new class of important materials that are increasingly being developed for use in research and health-related applications. Although the \textit{in vitro} antibacterial activity and efficacy of bulk zinc oxide material have been investigated, knowledge about the antibacterial activity of ZnO nanoparticles is very deficient. The antibacterial activity of ZnO has been studied largely with different pathogenic and nonpathogenic bacteria such as \textit{S. aureus} and \textit{E. coli} [1, 2, 3, 4]. ZnO nanoparticles are believed to be nontoxic, biosafe, and biocompatible and have been also used as drug carriers, cosmetics, and fillings in medical materials. Several reports have addressed the harmful impact of nanomaterials on living cells, but relatively low concentrations of ZnO are nontoxic to eukaryotic cells [5-10]. ZnO nanoparticles significantly inhibit growth of a wide range of pathogenic bacteria under normal visible lighting conditions [2]. Several investigations suggest that different morphologies (particle size and shape) of ZnO have different degrees of antibacterial activities [2, 11-14]. Many researchers made different structured ZnO nanomaterial with different capping agent.

Here, different structured ZnO nanomaterial with increasing the molar concentration of the capping content 3-Mercaptobenzoic acid had been synthesized. It is an organic compound containing carboxyl and sulfhydryl functional groups. Fig1 describes the structure of the capping agent.
The preparation of different ZnO nanostructures by using the four different molar concentrations (0.05, 0.1, 0.2 and 0.3) had been investigated through single step solid state reaction. Solids do not react together at room temperature over normal time periods and it is necessary to heat them for reaction. The factors on which the feasibility and rate of a solid state reaction depends on reaction conditions are structural properties of the reactants, surface area of the solids, their reactivity and the thermodynamic free energy change associated with the reaction. Recently, Ye et al. [15] had explored an effective approach to synthesizing nanocrystals of oxides, sulfides, oxalates, and carbonates, etc. based on a one-step solid-state reaction. Jin et al. first employed this technique to synthesize ZnO nanorods by using the reagents of Zn(CH₃COO)₂ 2H₂O (Zinc acetate dehydrate) and NaOH as raw materials, and triethanolamine as the surfactant (stabilizer) [16]. They claimed that the product prepared is single-phase hexagonal ZnO derived from the X-ray diffraction (XRD) patterns. It is thus necessary to discriminate whether the final product of this solid-state reaction method is pure ZnO only or a mixture of ZnO and Zn(OH)₂ with a non-negligible content of the latter component. There is a possibility of the coexistence of Zn(OH)₂ in
the cases of using zinc acetate, NaOH with other stabilizers [17, 18]. In 2008 Yan Zhu et al. has prepared ZnO nanoparticles using the one-step solid-state reaction with ZnSO$_4$, NaOH as reagents, and noted in the final product the existence of a certain amount of Zn(OH)$_2$ [19]. Increasing the molar concentration of NaOH in the reagents is favorable for removing Zn(OH)$_2$ in the product, thus pure ZnO nanoparticles are obtained by this simple method.

Here 3-Mercaptobenzoic acid had been used as both the stabilizer and surface capping agent in the experiment. Simultaneously the surface capping agent 3-Mercaptobenzoic acid had helped in formation of various structures of ZnO nanomaterial from nanoparticles to rod like structure.

The objective of this study was to thoroughly examine the significance of 3-Mercaptobenzoic acid assisted ZnO nanostructures on bacterial cell wall destruction and the subsequent status of these nanomaterials after getting entered into the bacterial cell. Changing of the capping content concentration might lead to the change in appearance of different nanostructures from spherical particles to branched nanorods and the change in morphology showed different antibacterial activity for *E.coli* and *S.aureus* bacteria. Actually In this study, an attempt has been made to investigate the exact antibacterial activity mechanism behind this reported antibacterial property of these ZnO nanomaterials. Generally, the antimicrobial mechanism of chemical agents like 3-Mercaptobenzoic acid assisted ZnO nanostructures can be understood by studying the specific binding on the surface of the agent with the microorganism and the consequent mode of action against those bacteria. The carboxyl and sulphahydryl groups present in the 3-Mercaptobenzoic acid may lead to the bacterial cell surface attachment.
6.2 Experimental Procedure

The chemical reagents used in this work were Zinc acetate dihydrate, NaOH pellets and 3-Mercaptobenzoic acid with of analytical grade purity. In the solid-state reaction, Zinc acetate dihydrate (1 mol, 2.0 g) was ground for 5 min with NaOH (3 mol, 1.092 g) along with the stabilizer 3-mercaptobenzoic acid by using the four different molar concentrations (0.05, 0.1, 0.2, and 0.3) at room temperature. After the mixture was ground for 5 minutes, the product was washed many times with distilled water and alcohol to remove the by-products. The final product was then filtered and dried into solid powders at little warm condition for 2 h in air [19]. Several analytical methods were used to characterize the composition, morphology and structure of the final products. Finally antibacterial activity test was investigated through cup plate method against *E.coli* and *S.aureus* bacteria. The schematic diagram of synthesis process is as given in Fig2 as a flow chart.

![Schematic diagram of synthesis process for preparing 3-mercaptobenzoic acid embedded ZnO nanostructures](image-url)

*Fig2: Schematic diagram of synthesis process for preparing 3-mercaptobenzoic acid embedded ZnO nanostructures*
6.3 RESULTS AND DISCUSSION

6.3.1 XRD analysis of 3-Mercaptobenzoic acid assisted ZnO nanostructures

A typical XRD pattern of the synthesized product had been shown in Fig3. All the diffraction peaks coincide well match with the diffraction peaks from the hexagonal phase ZnO. The XRD peaks had corresponded to the (100), (002), (101), (102), (011), (103), (200), (112), and (201) planes of ZnO in the wurtzite structure (JCPDS card file No. 361451). No peaks for Zn or other impurities were detected in the diffraction data revealing the zincite phase of the products. The average crystallite size was 31.53 nm (0.05M), 31.72 nm (0.1M), 25.39 nm (0.2M) & 27.84 nm (0.3M) calculated by the Debye Scherrer’s formula. The d values were computed and compared with standard d values of ZnO, resulting in excellent matching which have been summarized in Table1.

![XRD diagram](image)

**Fig3:** XRD analysis of 3-Mercaptobenzoic acid assisted ZnO nanomaterial. **ZM1:** 0.1M capping content sample, **ZM2:** 0.2M capping content sample, **ZM3:** 0.3M capping content sample, **ZM4:** 0.05M capping content sample
6.3.2 UV-VIS spectroscopy analysis of 3-Mercaptobenzoic acid assisted ZnO

The optical transmission spectra of the capped ZnO nanostructures were taken in the range between 300nm to 1000nm in wavelength by using UV-VIS spectrophotometer (*Perkin Elmer*,

<table>
<thead>
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<th>Standard d value of ZnO</th>
<th>(hkl) 2</th>
<th>Computed d value ZM1</th>
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<td>(112)</td>
<td>67.86</td>
<td>(112)</td>
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</table>

*Table 1*: XRD analysis of 3-Mercaptobenzoic acid assisted ZnO nanomaterial compared with that of standard XRD analysis of ZnO.
lambda 35 UV-VIS spectroscopy). The UV-transmittion was shifted towards higher wavelength from 359 to 362 nm as the molar concentration of the capping content increased from 0.05 to 0.3 (Fig4). It had indicated as the molar concentration of capping content increases initially from 0.05 to 0.1, the band gap tends to decrease from 4.72 eV to 4.18 eV. With further increase of capping agent it had not found any further change in band gap. The band gap plotting for all capped ZnO nanostructures were revealed in Fig5.

**Fig4:** UV-VIS spectroscopy analysis of 3-mercaptobenzoic acid assisted ZnO nanostructures

**Fig5:** Band gap calculation of 3-mercaptobenzoic acid assisted ZnO
6.3.3 Morphology analysis of capped ZnO by SEM

From the SEM analysis the structure of the 3-Mercaptobenzoic acid capped ZnO nanomaterial was found to be almost spherical (Fig6A) with lowest capping content. It was retained its spherical nature after increasing the capping content from 0.05M to 0.1M (Fig6B). The morphology of nanomaterial was changed towards the preferential growth towards the rod like feature. The same behavior was further observed with 0.2M of 3-mercaptopbenzoic acid and it’s structure found to be perfect rod like shape (Fig6C). The structure of nanorods were altered by formation of branches at the conditions of 0.3M of 3-mercaptopbenzoic acid (Fig 6D). HRTEM images of these nanomaterials had confirmed the better understanding of the structural changes. The actual mechanism of the formation of these different structures was thoroughly explained by the diagrammatic form where the capping agent, 3-mercaptopbenzoic acid had attached & bound with the surface hydroxyl group of the ZnO. The already covered ZnO molecule by 3-mercaptopbenzoic acid bound another capped ZnO molecule with another tail of capping agent leading to branching. Finally all of these capped ZnO nanomaterial bound with one another to form the entire network like structure as shown in Fig7.

Fig6: SEM analysis of A) 0.05M 3-mercaptopbenzoic acid capped ZnO sample, B) 0.1M 3-mercaptopbenzoic acid capped ZnO sample, C) 0.2M 3-mercaptopbenzoic acid capped ZnO sample and D) 0.3M 3-mercaptopbenzoic acid capped ZnO sample.
6.3.4 Morphology analysis by HRTEM study

The morphology of the nanomaterials was further studied using transmission electron microscopy. TEM images had revealed the crystalline structures and resolved the exact morphology of the samples which was initially analyzed by SEM. Fig8 had showed TEM images of ZnO nanoparticles prepared by 3-Mercaptobenzoic acid as stabilizing ligands. The addition of 3-Mercaptobenzoic acid had changed the structure of the ZnO nanomaterial from spherical to branched rod pattern which was clearly revealed in the images. The molar ratio of ZnAc₂ to 3-
Mercaptobenzoic acid was maintained at 1:0.05. ZnO nanoparticles with the average particle size of ~40 nm were formed (Fig8A). The shape of the prepared nanoparticles were found to change with the increase of the molar concentration of 3 Mercaptobenzoic acid to 0.1. The initialization of the rod like morphology was confirmed due to interlocking of capped nanomaterials with one another (Fig8D). Further the increase of molar concentration of 3-Mercaptobenzoic acid to 0.2, had generated perfect nanorods features and these nanorods became branched after reaching the molar concentration to 0.3 (Fig8G and 8J). The size of the nanostructure was approximately 20-25 nm (diameter) for 0.1M capped sample, which had initialized the rod like structure. The nanorod like morphology was 100-120 nm in length & 20-30 nm in diameter whereas, the branched pattern had kept the length same as the previously prepared nanorod but there were appearances of tiny branches with 5-8 nm in diameter. The SAED pattern had showed the well-defined electron diffraction spots, confirming the single crystalline nature of the synthesized ZnO nanostructures. The SAED spot pattern had confirmed the hexagonal phase of the ZnO as computed from d values. The lattice fringes have calculated and found to be of 0.28 nm from the figure in HRTEM images, had showed the inter-planar spacing of the (100) lattice plane of ZnO hexagonal phase. This figure further revealed the structure of nanomaterial with intricate design and shape of nanomaterials. At the lowest capping content the entire surface of ZnO was found to be covered with the help of surface hydroxyl group. The no vacant space was left on the ZnO surface due to this capping. The binding property of the surfactant may leads to the preferential growth feature forming the one dimensional nanorod like feature due to their binding properties. At the final stage branching of nanorod like pattern had been found given in Fig8J in HRTEM images.
**Fig 8:** TEM analysis of **A)** 0.05M 3-mercaptopbenzoic acid capped ZnO sample, **D)** 0.1M 3-mercaptopbenzoic acid capped ZnO sample, **G)** 0.2M 3-mercaptopbenzoic acid capped ZnO sample and **J)** 0.3M 3-mercaptopbenzoic acid capped ZnO sample, **C), F), I), L)** SAED patterns of capped ZnO
6.3.5 Study of surface functionalization by FTIR

Fig 9 had exhibited the confirmatory FTIR spectra analysis for the obtained surface capped ZnO nanoparticles with 3-mercaptophenzoic acid. The main absorption band due to O-H stretching of hydroxyl group had observed at 3430 cm\(^{-1}\) for 3-mercaptophenzoic acid only whereas this absorption band had obtained at 3410 cm\(^{-1}\) for surface capped ZnO by the capping element (3-mercaptophenzoic acid). The FTIR spectrum of 3-MBA adsorbed on ZnO (Fig 9) was dominated by the strong band at about 1596 cm\(^{-1}\), which was assigned to c=c aromatic stretching of benzene ring, from 3-mercaptophenzoic acid respectively. The band at 1797 and 1718 cm\(^{-1}\) were due to the presence C = O, of only 3-mercaptophenzoic acid and surface capped ZnO respectively. The band at 1396 cm\(^{-1}\) was corresponded to the presence of C–O [20, 21]. The IR spectrum had showed a standard peak from zinc oxide nanoparticles in the range between 400-600 cm\(^{-1}\)[22]. The absorption band at 487 cm\(^{-1}\), 549 cm\(^{-1}\), and 565 cm\(^{-1}\) were assigned to the Zn-O stretching bond which was shifted from 487 cm\(^{-1}\) to 549 cm\(^{-1}\) and finally had reached at 565 cm\(^{-1}\) after increasing the molar concentration of surface capping content 3-Mercaptophenzoic acid. This shift was attributed to the change in particle size and morphology [23, 24, 25]. The FTIR result was summarized in the Table 2.

![Fig9](image)

**Fig9:** FTIR analysis of 3-Mercaptophenzoic acid assisted ZnO nanomaterial. **ZM0**-0.05M 3-mercaptophenzoic acid capped ZnO sample, **ZM1**-0.1M 3-mercaptophenzoic acid capped ZnO sample, **ZM2**-0.2M 3-mercaptophenzoic acid capped ZnO sample and **ZM3**-0.3M 3-mercaptophenzoic acid capped ZnO sample.
6.3.6 Optical property study by Photoluminescence spectroscopy analysis

The optical property of the ZnO nanostructures was characterized by the PL spectra measurement at room temperature. Figure 10 had showed the PL image analysis of surface capped ZnO nanomaterial. It had indicated that the PL spectrum of ZnO nano crystal was mainly composed of two emission bands. One was ultraviolet emission (UVE) band and the other was deep-level emission (DLE) band. The DLE in ZnO was considered to be related to intrinsic defects such as oxygen vacancy eVOT or Zn interstitial eZnIT [26, 27]. UV emission at 378 nm was observed for lowest capping content sample. The slight shift of the emission peak to higher wavelength i.e 384 nm was due to the increased size effect [28]. As shown in curve, the PL

<table>
<thead>
<tr>
<th>ZnM0</th>
<th>ZnM1</th>
<th>ZnM2</th>
<th>ZnM3</th>
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<td>Vibrational modes</td>
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<td>3418</td>
<td>O-H stretching</td>
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<td>de</td>
</tr>
<tr>
<td>1994</td>
<td>c=O symmetric stretching of benzene ring</td>
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<tr>
<td>1188</td>
<td>C=O</td>
<td>de</td>
<td>de</td>
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<tr>
<td>407</td>
<td>Absorption of ZnO bad</td>
<td>540</td>
<td>Presence of ZnO bond</td>
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</tbody>
</table>

| 3430   | O-H stretching |
| 1797   | C=O          |
| 1496   | C-O          |
| 1421   | C=C          |

Table 2: FTIR analysis in the table form
spectrum of surface capped ZnO had a great change when molar concentration of surface capping element increased to 0.1M. The broad band centered at 410nm and in the range between 500-600 nm [29, 30]. The UV emission had corresponded to exciton recombination related near-band edge emission and the deep level visible emission had originated from the localized levels in the band gap [30] (Fig 10). The visible emission was considered to originate from the electron transition from a singly ionized oxygen vacancy to the photoexcited hole [31]. The violet emission of the photoluminescence (PL) spectra of the nanoparticles had been observed with increasing capping content. The lowest capping content ZnO nanoparticles did not show any violet emission; but when the molar concentration of the capping content increased to 0.1, it had exhibited a remarkably violet band. ZnO branched nanorod structure might lead to the appearance of multiple resonances what modulate the photoluminescence spectra at 410 nm wavelength. As shown in Fig10, ZnO branched nanorod had provided cavities so that signal could circulate and strengthened in them [32, 33]. In all samples it was found that the band edge emission was much higher in intensity than the deep level emission, suggested the positive role of capping. One of the probable reasons might be due to the surface oxygen vacancies as the defects that gave rise to the violet and green emission.

![Fig10: PL analysis of 3-Mercaptobenzoic acid assisted ZnO nanomaterial. ZM0-0.05M 3-mercaptobenzoic acid capped ZnO sample, ZM1- 0.1M 3-mercaptobenzoic acid capped ZnO sample, ZM2- 0.2M 3-mercaptobenzoic acid capped ZnO sample and ZM3- 0.3M 3-mercaptobenzoic acid capped ZnO sample.](image-url)
6.3.7 The effect of 3-Mercaptobenzoic acid assisted different ZnO nanostructures on E.coli (Gram-Ve) and S.aureus (Gram+Ve) and its Antibacterial activity

6.3.7.1 Sample preparation for antibacterial activity study

Both E.coli and S.aureus bacteria were incubated at 37°C, under continuous shaking at 120 rpm. At the exponential phase bacteria were harvested by centrifugation at 4000g for 10 min at 4°C followed by washing twice with 10mM phosphate buffer saline (PBS, pH 7.2). The bacteria were suspended in PBS. Separately, 0.02 gm of both 3-Mercaptobenzoic acid assisted ZnO spherical nanoparticles and branched nanorods were mixed in the bacterial medium & were incubated under same condition. At the exponential phase, the bacteria with nanomaterials were harvested individually. Finally the bacteria attached to both 3-Mercaptobenzoic acid assisted ZnO spherical nanoparticles (0.05M) and branched nanorods (0.3M) were dried in vacuum drier.

Tests for assessment of antibacterial activity;

6.3.7.2 Study of attachment of 3-Mercaptobenzoic acid assisted ZnO spherical nanoparticles and ZnO branched nanorods with both E.coli and S.aureus bacterial cell by FTIR

Fig11 had revealed the comparative FTIR spectral analysis of untreated E.coli, S.aureus and both the bacteria treated with sample (MBA capped ZnO). The absorption peak at 1084 cm⁻¹ was typical P=O stretching in peptidoglycan layer of E.coli and S.aureus whereas the band at 3316 cm⁻¹, 3327 cm⁻¹ were reflected stretching vibrations of O-H and NH (the functional group in polysaccharides and proteins). Meanwhile, the band at 1678 cm⁻¹, 1651 cm⁻¹ were the result of C=O and C-N (Amide I) stretching, and 1583 cm⁻¹, 1557 cm⁻¹ had reflected a combination of N-H bending and C-N (Amide II) stretching (Amide I and Amide II) for E.coli and S.aureus [34, 35, 36]. This was due to the functional groups in proteins for the bacteria. After treatment with
MBA capped ZnO nanomaterials (with the four different morphology), the band relative to those observed in untreated cells were shifted to lower value. The relative shift of these bands had suggested that polysaccharides and proteins of the bacterial cells were involved in the attachment which might caused the ultimate destruction of bacterial cell. For MBA capped ZnO nanostructures the band at 1084 cm$^{-1}$, which assigned the P=O stretching in peptidoglycan layer of E.coli had shifted to 1077 cm$^{-1}$, 1093 cm$^{-1}$, 1101 cm$^{-1}$ and 1110 cm$^{-1}$ respectively for spherical nanoparticles, attached spherical nanoparticles, nanorods and branched nanorods. Likewise the bands at 1678 cm$^{-1}$ & 1583 cm$^{-1}$ (Amide I and Amide II) were also shifted to 1638 cm$^{-1}$ & 1542 cm$^{-1}$; 1685 cm$^{-1}$ & 1558 cm$^{-1}$; 1662 cm$^{-1}$ & 1551 cm$^{-1}$; and 1669 cm$^{-1}$ & 1534 cm$^{-1}$ respectively. The attachment was materials structures dependent. The FTIR analysis had confirmed the interaction of these nanoparticles with E.coli bacteria [37].

![FTIR analysis](image)

**Fig11:** FTIR analysis of 3-Mercaptopbenzoic acid assisted ZnO nanomaterial attached with E.coli: ZM0-0.05M 3-mercaptobenzoic acid capped ZnO sample, ZM1- 0.1M 3-mercaptobenzoic acid capped ZnO sample, ZM2- 0.2M 3-mercaptobenzoic acid capped ZnO sample and ZM3- 0.3M 3-mercaptobenzoic acid capped ZnO sample.
In case of *S. aureus*, the all bands assigned by P=O stretching in peptidoglycan layer, O-H & NH (the functional group in polysaccharides and proteins) stretching and Amide I, Amide II stretching shifted to lower value but this shifting was not as much blue shift as in the case of *E. coli*. The FTIR analysis had confirmed the interaction of these nanoparticles with *S. aureus* bacteria and the FTIR results had confirmed that the interaction with *E. coli* was stronger than the *S. aureus* (Fig12).

**Fig12:** FTIR analysis of 3-Mercaptobenzoic acid assisted ZnO nanomaterial attached with *S. aureus*. ZM0-0.05M 3-mercaptobenzoic acid capped ZnO sample, ZM1- 0.1M 3-mercaptobenzoic acid capped ZnO sample, ZM2- 0.2M 3-mercaptobenzoic acid capped ZnO sample and ZM3- 0.3M 3-mercaptobenzoic acid capped ZnO sample.
6.3.7.3 Study of attachment of 3-Mercaptobenzoic acid assisted ZnO spherical nanoparticles and ZnO branched nanorods with both E.coli and S.aureus bacterial cell by TEM

TEM image of typical E.coli bacteria and the 3-Mercaptobenzoic acid assisted ZnO spherical nanoparticles (0.05M) and ZnO branched nanorods (0.3M) attached to the cell surface were studied. TEM image of E.coli bacteria (Fig13) had clearly revealed that typical E.coli bacteria had rod like structure with intact cell membrane denoted by glossy white colour at external cell surface while dense cell cytoplasm inside the cell appeared dark black. Attached image had corroborated the SAED pattern of the bacterial structure and this pattern had been confirmed the amorphous nature of bacterial cell. Fig13B & 13D had depicted the TEM image of E.coli bacteria treated with 3-Mercaptobenzoic acid assisted ZnO spherical nanoparticles (0.05M) and ZnO branched nanorods (0.3M). TEM micrographs of E. coli grown in the presence of functionalized ZnO samples (for spherical nanoparticle) had showed preliminary results of cellular internalization and cell wall disorganization. The E.coli bacterial cells were destructed after nanomaterial treatment. It had revealed dramatic changes in the structure of the cell wall & in the nature of the entire cell content. The outermost cell membrane layer was destroyed and the cytoplasm was no longer uniform. It came out and piled up in selected region of the cell forming patches interspersed with empty spaces [37, 38]. Moreover, it was demonstrated, that 3-Mercaptobenzoic acid assisted ZnO spherical nanoparticles (0.05M) can damage the E.coli cell wall through cell internalization process which had been confirmed by the SAED pattern. The dotted ring of the SAED pattern had confirmed the presence of ZnO nanocrystal inside the amorphous E.coli cell; which had been clearly revealed the internalization process.

In case of ZnO branched nanorods (0.3M) the same internalization mechanism was observed similar to the spherical nanoparticles but the cell membrane disorganization was higher for these branched ZnO nanorods. The antibacterial activity test through cup-plate method had
been already revealed the same information i.e. branched ZnO nanorods had exhibited the higher inhibition zone from spherical ZnO nanoparticles (discuss later). Moreover, it was demonstrated, that 3-Mercaptobenzoic acid assisted ZnO branched nanorods (0.3M) can damage the E.coli cell wall through cell internalization process which had been confirmed by the SAED pattern (Fig13D). The dotted ring had been shown from the SAED pattern, which had confirmed the presence of ZnO nanocrystal inside the amorphous E.coli cell and had indicated the internalization process.

**Fig13:** A) Typical E.coli bacteria shows intact cell membrane with intact cell cytoplasm; attached SAED pattern shows amorphous nature of bacteria, B) Cell internalization process through 3-Mercaptobenzoic acid assisted ZnO spherical nanoparticles (0.05M) with bacterial cell disorganization, C) higher magnification TEM analysis shows crystalline nanoparticles inside the bacterial cell and observed attached SAED pattern confirms the presence of this crystalline nanoparticles inside it, D) cell internalization process through 3-Mercaptobenzoic acid assisted ZnO branched nanorods (0.3M) and observed attached SAED pattern confirms the presence of this crystalline nanoparticles inside the bacterial cell.
The peptydoglycan layer is much thicker in case of \textit{S.aureus}. Fig14A had showed the exact spherical structure of this bacterial cell. The cell had been destructed after getting attached with 3-Mercaptobenzoic acid assisted ZnO spherical nanoparticles (0.05M) and branched nanorods (0.3M) respectively (Fig14B & 14C). Cell internalization process had also observed for gram positive bacteria i.e \textit{S.aureus}.

![Fig14](image)

**Fig14:** A) TEM image of typical \textit{S.aureus} and inset SAED pattern confirms amorphous nature. B), C) 3-Mercaptobenzoic acid assisted ZnO spherical nanoparticles (0.05M) and branched nanorods (0.3M) attach with \textit{S.aureus} and observed cell internalization process and inset confirms the presence of crystalline ZnO through SAED pattern.

### 6.3.7.4 Antibacterial activity test through zone of inhibition study; Kirby-Bauer test (Cup plate method)

Gram-negative bacteria \textit{E. coli} and Gram-positive bacteria \textit{S.aureus} were used for the investigation of antibacterial activity. Culture broth (Nutrient broth from HIMEDIA) and culture agar (Nutrient agar from HIMEDIA) were used as culturing nutrient. Both \textit{E. coli} and \textit{S.aureus} were grown separately at 37 °C in an incubator. The antibacterial effects of uncapped ZnO, 3-Mercaptobenzoic acid assisted two different ZnO nanostructures with amoxicillin were also investigated.
Antibacterial effect of 3-Mercaptobenzoic acid assisted ZnO nanomaterials had been analyzed by the study of inhibition zone in agar plate with respect to known antibiotic Amoxicillin. All tests have been repeated three times after culture incubation at 37°C overnight and the average zone diameter were given in Table 3 which had revealed the antibacterial zone due to the presence of known antibiotic (amoxicillin) and different structured ZnO nanomaterial respectively. From the table it was confirmed that larger inhibition zone created by branched ZnO nanorod. As the capped ZnO sample had changed their structure with increasing molar concentration of the capping content, the inhibition zone had increased and it confirmed the different structural effect of 3-Mercaptobenzoic acid assisted ZnO nanomaterial which was responsible for different antibacterial activity and the behavior of branched nanorod morphology had been revealed the better result.

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<tr>
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<td>~19.98</td>
</tr>
<tr>
<td>ZM2 (nanorods)</td>
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<td>~20.30</td>
</tr>
<tr>
<td>ZM3 (branched nanorods)</td>
<td>~22.50</td>
<td>~21.00</td>
</tr>
</tbody>
</table>

Table 3: Comparative analysis of inhibition zone diameter due to 3-Mercatobenzoic acid assisted different structured ZnO nanomaterial
6.4 Conclusion

Here, sulphahydryl functional groups have been successfully introduced to ZnO nanomaterials through 3 mercaptobenzoic acid encapsulation. This 3- mercaptobenzoic acid containing groups helped the attachment. The X-ray diffraction (XRD) patterns had confirmed the presence of pure single crystalline ZnO nanomaterials. No impurity association had been observed due to the capping. FTIR study had revealed the formation of ZnO nanomaterials with the attachment of surface functional groups. Here in this study an innovative technique had been developed. The molar concentration of the capping agent had been changed which was found responsible for the structural modification of ZnO nanomaterials and were characterized by SEM and HRTEM analysis.

Finally the antibacterial efficacy of capped nano ZnO had been investigated for the *E.coli, S.aureus* bacteria and at the same it had been trying to find out the probable mechanism behind the antibacterial effect. Both the spherical nanomaterials & branched nanorods have showed good antibacterial result against the both *E.coli & S.aureus* bacteria. The morphological changes of bacteria had been identified by TEM due to effect of 3- mercaptobenzoic acid assisted ZnO nanomaterials. Cellular internalization had occurred in case of both samples. The SAED pattern had confirmed the presence of crystalline ZnO nanostructures inside the cell. The antibacterial activity was observed through zone of inhibition study and it had indicated that 0.3M capped branched ZnO nanorod sample had showed the higher zone of inhibition for both *E.coli & S.aureus* bacteria.
6.5 References


[31] Lili Wu a, Youshi Wu a, Xiaoru Pan b, Fanyuan Kong b Optical Materials 28 (2006) 418–422